A preliminary phylogenetic study of *Copidosoma* spp. (Hymenoptera: Encyrtidae) associated with Noctuidae (Lepidoptera) based on 28S rDNA D2 sequence

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Abstract: Phylogenetic relationships amongst *Copidosoma* spp., *e. g. C. floridanum*, *C. primulum*, *C. truncatellum* and *C. agrotis* (Hymenoptera: Encyrtidae) associated with noctuid hosts (Lepidoptera) are inferred from nucleotide sequences of the D2 region of 28S rDNA. Both maximum-parsimony and maximum-likelihood analysis showed that *C. floridanum* and *C. primulum*, associated with Plusiinae and Heliothinae hosts separately, descend from a theoretical common ancestor, while *C. truncatellum* and *C. agrotis* associated with Noctuinae are more closer to each other. The D2 region of 28S ribosomal RNA appears potentially useful for understanding phylogenetic relationships in this genus.

Key words: Hymenoptera; Encyrtidae; *Copidosoma*; molecular phylogeny; 28S ribosomal RNA

1 INTRODUCTION

Ribosomal RNA (rRNA) gene sequences have been commonly used in taxonomic and phylogenetic studies of various hymenopterous taxa (Campbell *et al.*, 1993, 2000; Babcock and Heraty, 2000; Heraty and Polaszek, 2000; Hoy *et al.*, 2000; Babcock *et al.*, 2001; Manzari *et al.*, 2002; Pedata and Polaszek, 2003; Schmidt and Polaszek, 2007; Triapitsyn *et al.*, 2007). The large subunit 28S rDNA of eukaryotes includes several divergent domains (Hassouna *et al.*, 1984) or expansion regions (Hancock *et al.*, 1988) flanked by conserved core regions (Campbell *et al.*, 1993). It has been frequently used to infer phylogenetic affiliations of subgenera and distinguish sibling species of Chalcidoidea (Campbell *et al.*, 1993; Babcock and Heraty, 2000; De Barro *et al.*, 2000; Babcock *et al.*, 2001; Manzari *et al.*, 2002; Pedata and Polaszek, 2003; Schmidt and Polaszek, 2007).

Copidosoma (Hymenoptera, Encyrtidae) is a diverse and cosmopolitan group of 187 species with great economic importance (Hain and Wallner, 1973; Guerrieri and Noyes, 2005). In *Copidosoma*, *C. floridanum*, *C. primulum*, *C. truncatellum*, *C. agrotis* are morphologically similar and frequently misidentified. It is particularly true for *C. truncatellum* and *C. floridanum* (Noyes, 1988). All these species use Noctuidae (Lepidoptera) as host. The recorded hosts for *C. floridanum* are Plusiinae, while *C. agrotis* and *C. truncatellum* attack Noctuinae, and *C. primulum* parasites Heliothinae (Guerrieri and Noyes, 2005). *C. floridanum* was introduced into Hawaii in 1898 for biological control of *Chrysodeixis chalcites* (Esper) (Swezey, 1931). *C. primulum* (Mercet, 1921) (= *Litomastix heliothis* Liao) has been released in China against *Helicoverpa armigera* (Hübner) (Noctuidae) in wheat fields (Li *et al.*, 1996). Other than its potential as biocontrol agents, *C. floridanum* is of interest because it has been used as one of the models of polyembryonic studies (Strand, 1989; Grbic *et al.*, 1992). Despite the economic and scientific importance of the above *Copidosoma* spp., little is known about their phylogenetic relationships. The purpose of the present study is to give a phylogenetic relationship analysis of *C. floridanum*, *C. primulum*, *C. truncatellum* and *C. agrotis*, based on nucleotide sequences of the D2-28S rRNA gene. We do this work in order to understand the affinities between morphologically closed species and provide basic information for their use in biological control programs; to assess the relative usefulness of the

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D2-28S rRNA gene in future phylogenetic study of *Copidosoma* species.

2 MATERIALS AND METHODS

2.1 Insect specimens

Copidosoma samples used in this study, information on locations, host associations and GenBank accession numbers are detailed in Table 1. *Ageniaspis fuscicollis*, *C. boucheanum* and *C. cervius* are used as out groups. Voucher specimens were deposited in the Institute of Zoology, Chinese Academy of Sciences (IZCAS). The nucleotide sequences of *C. truncatellum* and *C. floridanum* already published by Gillespie *et al*. (2005) were downloaded from GenBank.

Table 1 Source of rRNA gene nucleotide sequence data used in the phylogenetic analysis

Species	Locations	Host associations	GenBank accession no.
Ageniaspis fuscicollis	Fujian, China	Yponomeutidae	EU856742
Copidosoma cervius	Shanxi, China	Geometridae	EU856744
Copidosoma boucheanum	Qinghai, China	Gelechiidae	EU856743
Copidosoma floridanum 1	Beijing, China	Noctuidae, Plusiinae	EU856748
Copidosoma floridanum 2	Guangxi, China	Noctuidae, Plusiinae	EU856749
Copidosoma floridanum 3		Noctuidae, Plusiinae	AY599319
Copidosoma truncatellum 1	Shanxi, China	Noctuidae, Noctuinae	EU856745
Copidosoma truncatellum 2		Noctuidae, Noctuinae	AY599320
Copidosoma agrotis	Shanxi, China	Noctuidae, Noctuinae	EU856746
Copidosoma primulum	Beijing, China	Noctuidae, Heliothinae	EU856747

2.2 DNA isolation, polymerase chain reaction (PCR) amplification and sequencing

DNA was extracted from the entire body of female adults. Total DNA was isolated and purified following procedures from the DNeasy Tissue Kit (Qiagen) and eluted in 200 μL of AE buffer. The forward and reverse primers were used for amplipying the D2 region of 28S rRNA gene: [F] 5′-CGT GTT GCT TGA TAG TGC AGC-3′ and [R] 5′-TTG GTC CGT GTT TCA AGA CGG G-3′ (Campbell *et al*., 1993). The cycling program was: denaturation step at 95℃ for 30 s, annealing for 45 s at 58℃, and extension at 72℃ for 1 min, with 32 – 35 cycles being performed. All PCR products were then purified and directly sequenced with the amplification primers. Sequencing was performed using the BigDye terminator v3.1 Cycle (ABI) and carried out with an ABI PRISM 3730XL sequencer.

2.3 Sequence alignment and phylogenetic analyses

Sequences were aligned using CLUSTAL X (Thompson *et al*., 1997). Final alignment was obtained manually. Aligned sequences were analysed with PAUP* version 4.0b10 (Swofford, 2002) using both the maximum-parsimony (MP) and maximum-likelihood (ML) methods. All characters were assigned equal weight and gaps were treated as missing characters. Bootstrap analyses were performed using PAUP* with 1 000 replicates and 20 random addition sequences. Distances between the sequences were also calculated based on the Hasegawa parameter (HKY85) using PAUP 4.0 b10.

3 RESULTS

The complete molecular data set includes 1 sequence of *Ageniaspis fuscicollis* and 9 sequences of *Copidosoma* spp. The aligned data set was 582 bases. All characters are of type 'unordered' and have equal weight. 426 characters are constant and 89 variable characters are parsimony-uninformative. 67 characters are parsimony-informative. With an exhaustive search, Parsimony analysis of the 28S-D2 data for all taxa resulted in a single tree (length 219, CI 0.863, RI 0.758). Importantly, the 28S-D2 gene region supports a clustering of different individuals or populations of each species, even when considerable sequence divergence is present, as in *C. floridnum* and *C. trucatellum*. Bootstrap values are generally high for apical clades (Fig. 1: A). The model selected by MODELTEST for the ML was (HKY + G), with the parameter of the gamma distribution (alpha) = 0.2957. The single ML tree had a -ln likelihood score of 1 853.09. For relationships, the ML tree (Fig. 1: B) was the same as that for parsimony.

Table 2 shows pairwise HKY85 parameter distances for all pairs of the 10 28S-D2 rDNA sequences. HKY85 distances for all 28S-D2 rDNA sequences ranged from 0.0057 to 0.1926. The levels of differentiation among *Copidosoma* spp. varied between 0.02527 to 0.17936. The levels of differentiation between *C. cervius* and the rest *Copidosoma* spp. varied between 0.16913 to 0.17936. The levels of differentiation between *C. boucheanum* and the rest *Copidosoma* species varied between 0.07540 to 0.09890. These levels were generally higher than those obtained with *C. floridnum*, *C. trucatellum*, *C. agrotis* and *C. primulum* which only ranged from 0.0252 to 0.07952.

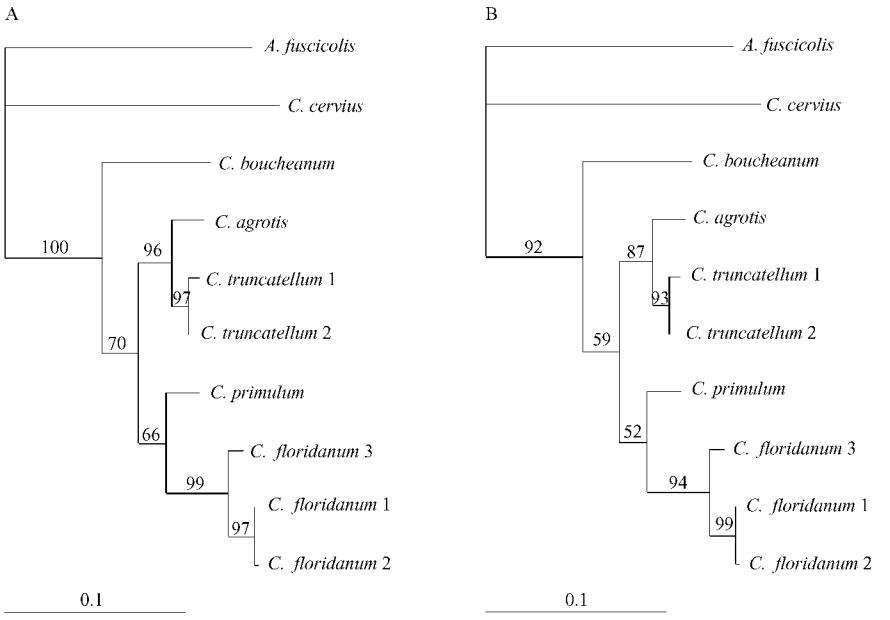

Fig. 1 Maximum parsimony (MP) tree (A) and maximum likelihood (ML) tree (B) from the analysis of the data, with bootstrap values (1 000 replicates) above the nodes.

Table 2 Pairwise HKY85-parameter distances for D2-28S rDNA sequences

	1	2	3	4	5	6	7	8	9	10
1. *C. truncatellum* 1										
2. *C. truncatellum* 2	0.00571									
3. *C. agrotis*	0.03087	0.02527								
4. *C. primulum*	0.04999	0.04604	0.05203							
5. *C. floridanum* 1	0.07258	0.06846	0.07943	0.05594						
6. *C. floridanum* 2	0.07266	0.06853	0.07952	0.05593	0.00189					
7. *C. floridanum* 3	0.06467	0.06258	0.07339	0.04794	0.02124	0.02121				
8. *C. boucheanum*	0.08097	0.07540	0.08746	0.08582	0.08945	0.08961	0.09890			
9. *C. cervius*	0.17936	0.17218	0.16913	0.17522	0.17273	0.17301	0.17463	0.17412		
10. *A. fuscicollis*	0.17241	0.16313	0.17181	0.17064	0.19251	0.19262	0.18918	0.17640	0.18749	

4 CONCLUSION AND DISCUSSION

In general, the relationships of species remained same in both the maximum-parsimony (MP) and maximum-likelihood (ML) analyses. Our data clarify the taxonomic status and the systematic relationships of these 4 closely related species or species groups. *C. floridanum* and *C. primulum*, associated with Plusiinae and Heliothinae hosts separately, descend from a theoretical common ancestor. While *C. truncatellum* and *C. agrotis* associated with Noctuinae are much closer to each other. Other than molecular data, some biological and morphological considerations support this arrangement. *C. truncatellum* and *C. agrotis* are 2 species that parasite cutworms and hepialids (Noctuinae) which deposited eggs at or near ground level, whereas the hosts of *C. floridanum* and *C. primulum* lay eggs well above ground level (Noyes, 1988). Genitalia in *C. truncatellum* and *C. agrotis* with phallobase narrowing proximally, digiti narrow and slender, parameres reduced; aedeagus apically bilaterally concave and pointed, with 2 buttonlike structure in apical third in addition to spermatic pores (Guerrieri and Noyes, 2005; Zhang and Huang, 2007).

Molecular tools provide new means of re-examining phylogenetic relationships and directions for the assessment of the phylogenetic value of morphological characters (Schmidt and Polaszek, 2007). For many Chalcidoidea and other Hymenoptera 28S-D2 appears to be good species marker, often demonstrating little within species variation, one basepair (bp) or less (Campbell *et al.*, 1993; Babcock and Heraty, 2000; Campbell *et al.*, 2000; De Barro *et al.*, 2000). This is also demonstrated in our data set. 28S-D2 shows little variation within *Copidosoma* species (0.00189 to 0.02121 in *C. floridanum*) but substantial sequence

diversity between closely related species of *Copidosoma* (see results above). In Aphelinidae, the 28S D2 region has been shown to be very promising for the investigation of phylogenetic relationships at the generic level (Babcock *et al*., 2001; Manzari *et al*., 2002; Schmidt *et al*., 2006; Schmidt and Polaszek, 2007). Our results showed that the 28S D2 region is very useful for the investigation of phylogenetic relationships at the species level in *Copidosoma*. However, additional work is necessary to enlarge the sampling of *Copidosoma* species and thus extend the applicability of the dataset.

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